The Cytoplasmic Inclusions of the Germ-Cells.

PART IV. NOTES ON THE DIMORPHIC SPERMATOZOA OF PALUDINA AND THE GIANT GERM-NURSE CELLS OF TESTACELLA AND HELIX.

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With Plates 25 and 26 and 21 Text-figures.

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Introduction.

In this paper, the fourth of this series, I have given an account of the origin of the peculiar atypic sperms of Prosobranchia, and I have also described the remarkable behaviour of the nurse- or yolk-cells of several Pulmonate Mollusca, a peculiar phenomenon hitherto unnoticed, and of importance in any discussion on the nature of the chromatin.

Part I.—On the Recognition of at least Two Separate Categories of Cytoplasmic Elements.

About twenty years ago Golgi (**19**) discovered in the nerve ganglion an intra-cellular network surrounding the nucleus, which he named "apparato interno reticolare." Independently and about the same time the famous Spanish observer Cajal also described this network. Subsequent work showed that this structure was not always in the form of a net, but might consist of branched or unbranched rods, granules, or curved semi-lunar bodies. This in itself did not come very far into the province of the zoologist, but the appli-

cation of Golgi and Cajal's silver nitrate methods and the method of Kopsch to glands and tissues from every part of the body of both vertebrates and invertebrates and to Protozoa showed that this network or its representative was present in every cell; furthermore, modern cytology shows that the Golgi apparatus is, morphologically and chemically, distinct from the mitochondria, and methods which show one category of cell-organs may not and often do not show the other. The technique of the Golgi apparatus has been treated elsewhere. Text-figs. 1–14 give some impression of the universal occurrence of the two categories of cell-bodies —mitochondria and Golgi apparatus; here are drawn nerve, thyroid, spermatogonial, pancreas, hepatic, gastric, protozoan (Monocystis), fat, epithelial (Descemet's) cells of various species, which are partly taken from my own preparations and partly from the figures of other workers. The morphology and histo-chemistry of this apparatus I have studied in another paper. In the oögenesis of several forms properly studied a remarkably developed Golgi apparatus is found; this is the subject of a further communication to be published by me. In the resting cell the Golgi rods or grains may lie upon the archoplasm with which they are generally associated (Text-figs. 1, 3, 4, 5, 6, 11, etc.), but in the prophases of mitosis these rods or grains become sorted out into two groups around the centrosomes as in Text-fig. 12 (upper). In the metaphase and other stages of mitosis the Golgi apparatus keeps around each aster as in Text-fig. 12 (lower), and so each daughter-cell gets one-half approximately of the rods, granules, or branched structures of the mother-cell. Be it noted that the rods do not split like chromosomes, but go over bodily to one or the other daughter-cell. Perroncito (**35**) calls this sorting out at mitosis "dictyokinesis,"[1] the dictyosome or dittosome being a Golgi rod. My chondrioplasts in snails and slugs (**15, 16**) are evidently Golgi apparatus, as I have been able to show by histo-chemistry and other sources of proof and experiment.

[1] δικτύον, a net.

Technique and Material.

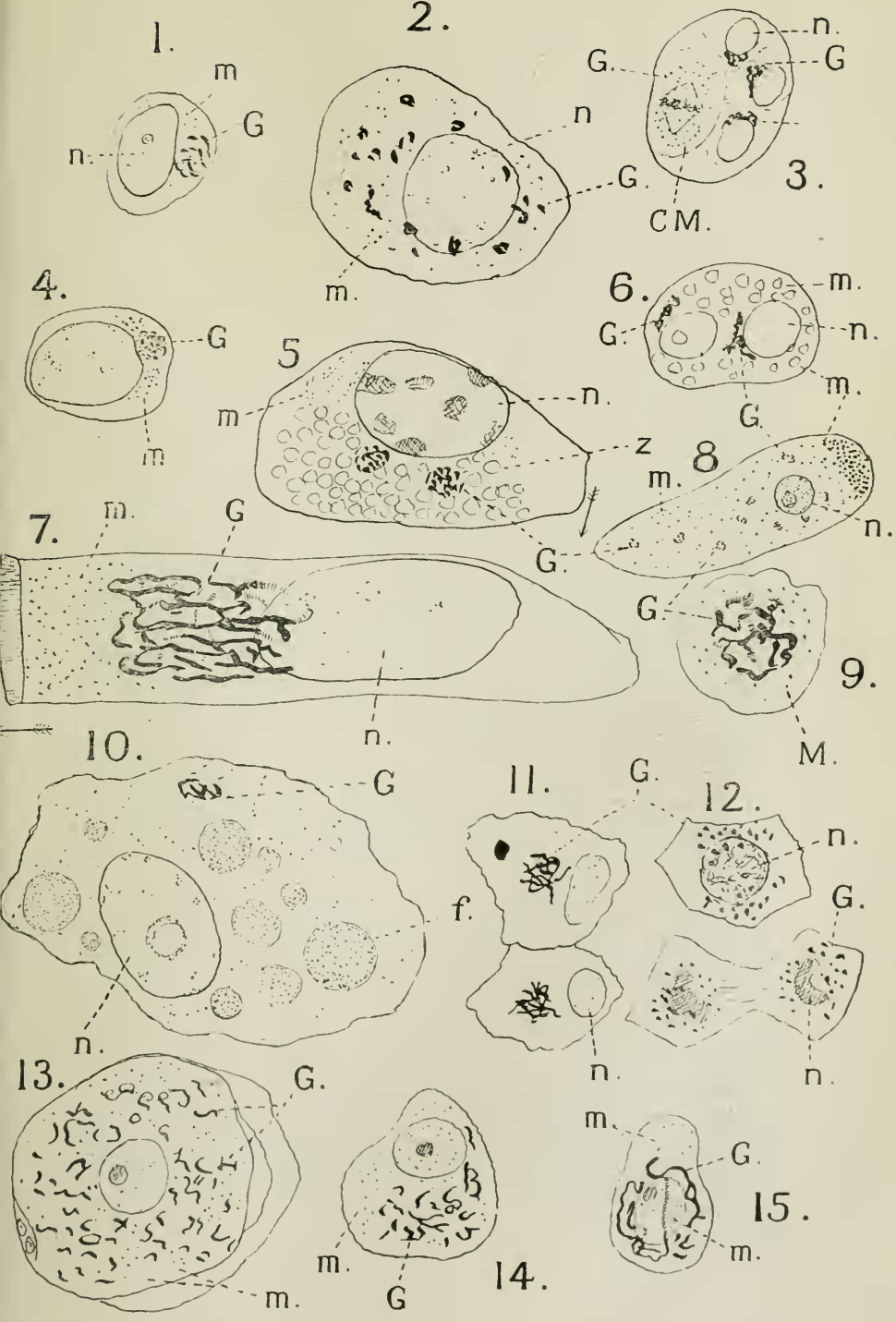
Paludina vivipara is quite common around Oxford. The snails were removed from their dish, the operculum drawn open, and the water in the shell shaken out; one drop of chloroform was placed on the head of the animal, and the operculum allowed to close. Immediately afterwards the upper whorls were broken with forceps, and the testis dissected out and cut into pieces about 4 mm. square, if the fixation was to be chrome-osmium.

I used the modification of Flemming without acetic acid, slightly diluted or strong. Also Champy's fluid in the same way just as described in my paper on Helix aspersa ('Quart. Journ. Micr. Sci.,' vol. 62, p. 563, last paragraph).

Besides the methods used in this previous paper, I used especially Kopsch's method (**25**); in this, fresh small pieces of gonad are dropped into about 6 cubic centimetres of 2 per cent. osmic acid in distilled water. They are left here in a dark place for fourteen days, after which they are washed overnight in running water and then through the alcohols—xylol—and embedded. As suggested by Hirschler (**23**), such sections stain well after this method in Altmann's acid

Description of Text-figs. 1–15.

Figs. 1, 2, 4, 5, 7, 8, 9, 10, 13–15, by Kopsch's method: Figs. 3, 6, 11 and 12, by formol silver-nitrate method of Cajal or Golgi; Figs. 1, 2, 4, 5, 7, 9 and 10, original. Fig. 1.—Cerebellar nerve-cell of Bufo vulgaris. Fig. 2.—Spinal ganglion cell of B. vulgaris. Fig. 3.—Thyroid epithelial cell of rat, showing Golgi apparatus in resting cells and one in mitosis (Cajal). Fig. 4.—Spermatogonium of Molge vulgaris. Fig. 5.—Pancreas-cell of M. vulgaris. Arrow points to acinar lumen. Fig. 6.—Liver-cells of rat, after Pappenheimer. Fig. 7.—Intestinal cell of M. vulgaris. Fig. 8.—Monocystis ascidiæ (Hirschler). Fig. 9.—Intestinal cell of Molge, cut transversely across Golgi reticulum. Fig. 10.—Fat-cell of Molge. Fig. 11.—Descemet's membrane cells in rest. Fig. 12.—Same in mitosis (dictyokinesis); compare fig. 3 (Deineka). Figs. 13 to 15.—After Cowdry; nerve-ganglion cells of pigeon, showing diffuse (fig. 13), excentric (fig. 14) and circumnuclear (fig. 15) arrangement of Golgi apparatus. Letters as follows: *G.* = Golgi apparatus. *C. M.* = cell in mitosis. *f.* = fat. *n.* = nucleus. *M.* = mitochondria. *z.* = zymogen granules.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fuchsin-picric acid. The preservation of Kopsch sections is often atrocious, but the method impregnates very intensely the so-called Golgi apparatus of histologists, known to zoologists as "Nebenkern" batonettes, chondrioplasts, dictyosomes, etc. (**2, 4, 9, 13, 15, 33**).

The Dimorphic Spermatozoa of Paludina vivipara.

In the following description I have clearly shown for the first time that the two series of spermatozoa of P. vivipara originate from cells whose mitochondria are either finely granular, or are few and banana-shaped, or more rarely very large and spherical. From the first sort of cell, with finely granular mitochondria, originate giant or atypic sperms; from the second sort, with banana-shaped rods, originate the typic or ordinary spermatozoa. I have identified positively such cell series back to the primary spermatogonia, but, though I have advanced strong evidence for believing that the germinal epithelial cells are also of two sorts, further work is being undertaken on the embryonic gonad in order to settle the question definitely.

Meves, in his latest paper on Paludina, has failed properly to describe the difference between the atypic and typic spermatogonia and spermatocytes. As will be seen, these differences constitute one of the most remarkable facts in our knowledge of the mitochondria in gametogenesis—facts which are all the more noteworthy because we have hardly any clue as to why such differences should exist in cells closely associated in one organ (Meves, 'Arch. f. mikr. Anat.,' Bd. lxi).

The Typic Spermatogenesis of Paludina vivipara.

The germinal epithelium in Paludina is very difficult to understand properly, because, in the differentiated gonad, the epithelium has fewer cells, and regions of proliferation are scantier than in Helix. The yolk or nurse-cells are present, but fairly small, and they are much flattened against the layer

of Ancel; their yolk also is not remarkably well developed. Germinal epithelial cells are few in number, and nearly always covered over by a part of a yolk-cell, or embedded in a yolk-cell (Pl. 25, fig. 13, *Y.*, yolk-cell). The walls of the testis are very scantily covered when compared with the richly-provided alveoli of a Helix or Testacella ovotestis. The germinal epithelium is not a syncytium, its cell elements being discrete.

In Pl. 25, figs. 13 and 14, are drawn two typical germinal epithelial cells; these are not at all easy to find, but they are extremely characteristic, and easily identified when once discovered. Such cells contain a slightly polymorphic nucleus, staining somewhat palely; there are generally two or more net-knots or karyosomes, though the nuclear reticulum is often hardly demonstrable.

In the cytoplasm it is generally possible to identify a mass of mitochondrial matter formed apparently of a number of rods, either separate or closely clumped together.

It is a very difficult matter to get cells in which the rods show so well as in Pl. 25, figs. 13 and 14, these cases being the best fixed and stained in a preparation.

It is quite impossible to count the number of these mitochondrial rods; they are never straight, but are nearly always S-shaped or even more sinuously twisted. In Pl. 25, fig. 14, the rod *M.R.X.* was easily drawn in with a camera lucida, and the presence of these structures I think to be indisputable.

Such primary male cells give rise by indirect division to large bunches of secondary spermatogonia; the diploid chromosome number appears (fourteen, as in Pl. 25, fig. 16), and a series of rapid divisions ensues. If a group of such cells at this stage be examined, it will be found that in the cytoplasm the mitochondrial rods have unravelled, and now form a number of separate, confusedly bent structures, as in Pl. 25, fig. 16, *M.R.* Every cell contains numbers of these rods, and by gently focussing up and down, a rod can be followed at least part of its way through the cytoplasm.

Pl. 25, fig. 16, is very typical, and illustrates how difficult it is to learn anything with regard to the number and mode of disposal of the mitochondrial rods. Such cells, after a series of divisions, enter growth; a reticulum appears in the nucleus, and this soon breaks into a number of faintly-staining chromatin loops; in this state the nucleus grows. After anaphase of the secondary spermatogonial division, the rods become grouped towards one side of the nucleus—as will be seen later, really around an archoplasmic zone, which has embedded on its surface Golgi rods (chondrioplasts, Nebenkern batonettes, dittosomi) (see Pl. 25, figs. 1 and 2, *A.R.*). The mitochondrial rods at this stage are much thicker shorter, and they are crescentic or U-shaped. There appear to be more than six or seven in every case I could see plainly enough to make an estimate. In Pl. 25, fig. 2, is drawn a view of the end of a cell such as that in Pl. 25, fig. 1; the rods are stumpy, all U-shaped, with their free ends generally towards the observer, and I was able to count at least thirteen.

Another element which was quite plain directly after anaphase was the archoplasm and the chondrioplasts; these were possibly eight in number, though it was very difficult to make a certain count. In the growth stage the mitochondrial rods constantly occupied the position indicated in Pl. 25, figs. 1 and 3. As has already been mentioned, the nucleus, from a very early stage, contained a number of loops. Soon these begin to form the synaptene stage, as has already been described by Meves. The loops pairing become resolved into chromosomes, as in Pl. 25, fig. 4, *C.H.* In no case did I find the spermatocyte reach a greater comparative size than that drawn in Pl. 25, fig. 3 (compare the atypic spermatocyte in Pl. 25, fig. 20).

I feel certain that at the prophases of the maturation mitoses the mitochondrial rods lengthen, so as to be as long as those drawn in Pl. 25, fig. 4 (compare this with the previous figure). The first spermatocyte division, seen as a polar view in Pl. 25, fig. 4, is drawn from the side in Pl. 25, fig. 5. The

irregularity of disposition of the mitochondrial rods is very evident; they meander over and around the mitotic figure in such a complicated manner that to count their number accurately was impossible; it is extremely difficult to tell where one rod begins and where it ends. In Pl. 25, fig. 4, there were at least seven rods, in Pl. 25, fig. 5, apparently nine. I am unable positively to say how the rods are disposed in division—that is to say, whether or not they are always divided in half at each division.

In Pl. 25, fig. 6, is a second maturation division at telophase; in all such stages it was impossible to count the rods in a satisfactory manner—I thought there were at least seven in this cell. I feel certain that at this stage they were not disposed regularly, so that one half the rod was in one cell, the other half in the other daughter-cell. In a later stage examination of the testes always revealed that at the latter end of telophase some of the mitochondrial rods were divided, as shown in Pl. 25, fig. 7, *C.M.R.*, by a constriction at the equatorial plate. I found such figures in both first and second spermatocyte divisions. If the cell in telophase was examined from the end as in Pl. 25, fig. 10, it was often possible to count four rods. In this figure some idea of the difficulty of counting the rods can be gleaned; the rods, *M.R.Z.* and *M.R.Y.*, are so twisted that it would be impossible to count them from such a cell viewed from the side; by focussing down one also came to the rod *M.R.X.*, and another above, but it should be pointed out that I was unable to say whether, for example, rod *M.R.Y.* was separate from or intercontinuous with rod *M.R.X.*

In fact I am convinced that to make certain as to the behaviour of these rods one must examine a more favourable species. In the spermatid (Pl. 25, fig. 8) one can often find a cell where the rods are shortening, and, though still bent, can be counted easily. Their number is nearly always four; the archoplasmic mass and the chrondrioplasts become evident after division, and lie beside the nucleus.

Concurrent with changes in the nucleus of the spermatid

heralding the formation of the spermatozoon, the mitochondrial rods become at first banana-shaped, then ovoid, and then quite spherical. Nearly always there are four—rarely five or six. I have never found fewer than four.

Spermateleosis in the Typic Spermatogenesis.

After the contraction of the mitochondrial rods to form the spheres it is very easy to count their number even with a $\frac{1}{6}$th objective. In 99 per cent. of cases the number is four, in 1 per cent. it is either five, six, or seven (Text-fig. 20).

The spheres are arranged almost always as in Pl. 25, fig. 9, and they soon fuse to form a solid mass. This mass elongates as in Pl. 25, figs. 11 and 12, to form the mitochondrial tail of the typic spermatozoon. No portion appears to be sloughed off, but the archoplasm with its two or three chondrioplast rods is rejected. In Pl. 25, fig. 25 A, is drawn the adult typic spermatozoon at half the scale of the other figures of the typic spermatogenesis; from $M.^1$ to $M.^2$ is the mitochondrial part; in front is the spirally twisted nucleus ($N.$); behind the region $M.^2$ the axial flagellum bare of mitochondrial matter.

With regard to the formation of the acrosome I was able to ascertain several facts; in Text-fig. 18 is drawn a part of a group of spermatids just after the fusion of the four or five mitochondrial spheres to form the macromitosome.

It will be seen that in front of each nucleus is adhering the archoplasm + chondrioplast apparatus, and apparently sticking into this is the acrosome. Each archoplasmic mass has, as far as one can see, two chondrioplasts (Golgi rods).

In Text-fig. 19 another drawing of this stage is given at a higher power. Apparently this relationship between nucleus and archoplasm is of short duration; afterwards the archoplasm drifts downwards as shown in Pl. 25, fig. 12 *AR*.

Note on Formation of Acrosome in Paludina and other Forms.

In Lepidopterous insects I showed that certain rods (Golgi apparatus?) found in the spermatocyte and spermatid swelled out to form spheres, and that it was from the latter that the acrosome appeared to be formed. Re-examination of new and of my old sections reinforces me in my opinion. Apparently the acroblasts of Lepidoptera are homologues of the chondrioplasts of Mollusca, and possibly of the inner Golgi network of the nerve cell.

There is now the evidence of Schitz (**42**) and of myself that in Molluscs the acrosome is formed somehow from the archoplasmic body upon which lie the chondrioplasts. Schitz prefers to believe that the acrosome in Columbella (**42**) is formed from a "graine siderophile" which is really one of the centrosomes.

It is to be noted that in Paludina and Columbella, which are Prosobranchs, there is only one centrosome which has been identified with certainty—that immediately behind the nucleus. In Pulmonata there are two, as explained by me in my work on Helix, but in Pulmonates it seems from certain stages that the acrosome is formed like that of Paludina and Columbella; how, then, could the "graine siderophile" of Columbella be the centrosome, when in Helix and other Pulmonates the two centrosomes have been otherwise accounted for, and yet the acrosome is formed in association with the archoplasm-Golgi apparatus as seems to occur in Paludina and Columbella? It appears that Schitz is wrong in interpreting his "graine siderophile" as a centrosome.

The Atypic Spermatogenesis of Paludina vivipara.

In not all the epithelial cells are the mitochondria rod-shaped. They are generally hard to distinguish in any but the most perfect preparations, but it seems certain that there

are forms of epithelial cells whose mitochondrial apparatus is not formed of rods, but of granules. In such forms the nucleus and other cell elements are similar to those of the cells containing long mitochondria. I consider that the germinal epithelial cells are in the testis of two sorts, one giving rise to typic, the other to atypic spermatozoa.

In Pl. 25, fig. 17, I have drawn what I think is a cell which will ultimately give rise to atypic spermatozoa.

Such cells divide to give rise to secondary cells whose general appearance closely resembles the secondary spermatogonia of Helix (**15**).

In the germinal epithelial cells which I think give rise to the atypic series, there is always to be seen a large archoplasm which is, I believe, never so conspicuous in the cells containing rods. In Pl. 25, fig. 15, is drawn a secondary cell (secondary spermatogonium) containing an archoplasmic mass at *A.M.* with mitochondria on it. From this stage and ever afterwards there can be no doubt as to the differences between the atypic and typic series. In Pl. 25, fig. 18, is a later atypic spermatocyte just after entry into the growth stage. The archoplasm is now seen to be studded over with a number of Golgi rods (chondrioplasts), and around it are very many granular mitochondria (*G.M.*). A later stage is drawn in

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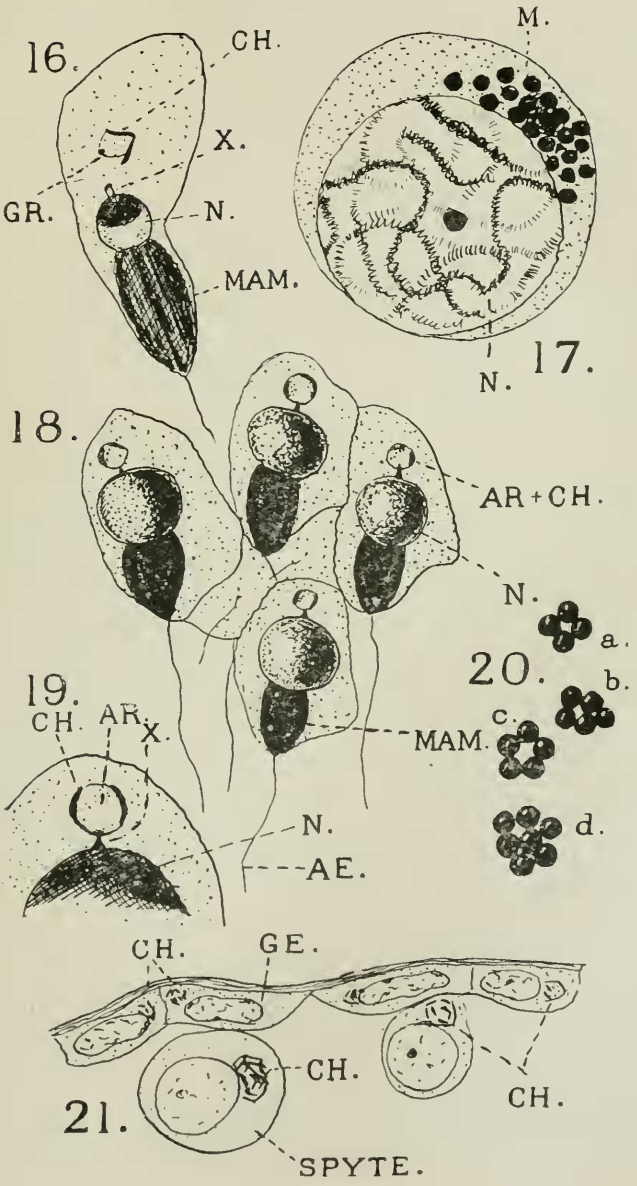
DESCRIPTION OF TEXT-FIGS. 16–21.

Fig. 16.—Spermatid of Columbella, to show formation of acrosome from a grain (*GR.*) embedded in the archoplasm (*CH.*). The grain will become stuck on to the protruding part of the nucleus at *X*. *MAM.* = macromitosome (mitochondrial). After Schitz (**42**). Fig. 17.—Typic spermatocyte of P. vivipara to show another form of mitochondria. Instead of rods the cell contained about twenty-six coarse spheres. Fig. 18.—Formation of the acrosome in P. vivipara. The archoplasm (and its chondrioplasts) is in each case lying in front of the nucleus, and, as is shown in the next figure (fig. 19), appears to be secreting the acrosome at *X*. *N.* = nucleus. Fig. 20, *a*, *b*, *c* and *d*.—Variations found in the mitochondrial spheres of the spermatid, just before they fuse to form the macromitosome, *MAM.* in fig. 18. *AE.* = axial filament. Fig. 21.—Germinal epithelium (*G.E.*) of Helix aspersa, prepared by Kopsch's method, to show Golgi apparatus (*CH.*) or chondrioplasts (Nebenkern) in indifferent cells and in spermatocyte (*SPYTE.*).

TEXT-FIGS. 16–21.



Pl. 25, fig. 19; this is a very characteristic cell; the archoplasm and its rodlets are very dense and around them are grouped the mitochondria, which are just like those of Pulmonates.

From this stage the cell does not generally grow very much, but in Pl. 25, fig. 20, is a larger specimen in the prophases of the first maturation division. The cytoplasmic inclusions have become grouped into two parts, evidently around the two centrosomes. The divisions which take place have been described at length by many authors (**26**, **30**, **39**). Meves may be consulted for a good description of the facts in Paludina, though personally I have not sought to examine thoroughly the nuclear or centrosomic phenomena during maturation of the atypic spermatocyte.

In Pl. 25, fig. 21, is a second maturation division of the atypic series; the mitochondria are scattered here and there in a haphazard manner, just as are the chromosomes.

Before passing on to the spermateleosis of the atypic cell it should be pointed out that the number of chondrioplasts in the typic spermatocyte is smaller than that of the atypic spermatocyte, while, as Meves showed, the archoplasm in the latter is the larger (compare Pl. 25, figs. 2 and 19).

The atypic spermatid contains approximately one-fourth of the mitochondria of the spermatocyte. Directly after telophase of the second maturation division the cytoplasmic inclusions are closely drawn around the centrosomic region to form a thick mass; the chromosomes do not all resume a reticulate shape, some appear to fuse, and generally two or more, rarely three, pale abnormal nuclei result (Pl. 25, fig. 22).

One of these nuclei becomes placed in front of the mitochondrial mass (*G.M.*) and the cell, then begins to metamorphose; the axial filaments (*FL*), as described well by Meves and others, now begin to grow out, and by a little later stage are quite long and like a tuft of hairs.

Just after the formation of the spermatid an examination of the cytoplasm reveals the fact that a faint, rather coarse

granulation is becoming evident. This granulation gradually becomes sharper in outline till the individual units are clearly defined. These grains correspond to Perroncito's "Mitochondria of Benda." In Pl. 25, fig. 23, these grains are clearly defined; the ordinary mitochondria at *G.M.* have collected to form a square mass, which later elongates with the growing filaments (*FL.*). In Pl. 25, fig. 24, the mitochondria have seemingly partially sloughed down the filaments in the region *AX*, which is nearly clear of them, to region *BX*, where they are still evident. The main bulk is at *G.M.* Perroncito's "mitochondria of Benda" are now quite evident, and the "nucleus" at *N.* is becoming somewhat shrunken and in parts more darkly staining. At *C.H.* is a mass formed mainly of rejected chromatin and chondrioplasts.

The final stages of spermateleosis in the atypic spermatozoon now take place; the "nucleus" in front shrinks, and if it does not altogether lose its previous character it at least changes greatly in size, shape and stainability.

The mitochondria which surround the multiple axial filamentary apparatus do not apparently slough off; at any rate, they are very difficult to discover in later stages. I can, however, say for certain that they take some part in the formation of the fully-formed atypic sperm, as some authors have already shown.

The axial filaments appear either to fuse, or at least to become so closely applied one to another as to cause the optical effect of a solid structure. In Pl. 25, fig. 25 B, the axial rod is shown stretching from the head to the tail, from which arise the brush of filaments; the so-called "mitochondria of Benda" have become very dense; it should be noticed that Pl. 25, figs. 25 A and B, are drawn at half the scale of the previous figures. Pl. 25, fig. 25 B, is drawn in optical section, the focus being brought on to the edge of the organism.

In Pl. 25, fig. 26, is drawn a part of the upper region of the atypic sperm to show the "Mitochondrialmantel" of Retzius at the same scale as the other figures on the plate.

The Golgi Rods or Chondrioplasts in the Atypic Spermateleosis.

Reference to Pl. 25, figs. 18–25, serves to show the fate of the Golgi apparatus in the atypic series spermateleosis. They finally slough off, as do those of the typic series, and I cannot find that they form part of either typic or atypic spermatozoon. I have examined ripe sperms in Kopsch's method in order to test this question. At no stage did I find an intercontinuous Golgi reticulum as drawn by Perroncito. The latter observer's work on the Golgi apparatus of Paludina is evidently biassed by his knowledge of Cajal's studies on the mammalian Golgi apparatus, where with formol-silver nitrate a proper reticulum seems demonstrable in nerve and other cells.

Possible Intermediate Forms Between Typic and Atypic Cells.

In some cases I have found a group of cells containing about twenty or thirty large spherical mitochondria as drawn in Text-fig. 17. These were much larger and fewer in number than the mitochondria of the atypic series, and I have no doubt that these cells were a variety of the form drawn in Pl. 25, figs. 1 and 2. The cell in Text-fig. 17 is a growing spermatocyte, but I never found round, coarse mitochondria like these in any other stage of spermatogenesis. Such mitochondria might elongate as they grow, but their main importance lies in the fact that they may constitute forms intermediate between atypic and typic spermatogonia and spermatocytes. In the Paludinas I examined such cells were rare.

Discussion.

In this paper I have shown that the well-known dimorphic spermatozoa arise from cells whose mitochondrial apparatus differs very remarkably. Possibly in no other animal is such a remarkable state of affairs existent. It has been established

that these remarkable differences between the cells of the two distinct series of spermatozoa are very early in origin, if not actually already present in the indifferent (undeveloped) germinal epithelial cells.

All the stages in the behaviour of the cytoplasmic elements of both typic and atypic cells have been followed out carefully for the first time. Meves spoilt his work by using a Flemming with acetic acid—a fault followed by Reinke ; while Perroncito treats with the Golgi apparatus almost exclusively, and his main object seems to be to establish the fact that Paludina has a distinct Golgi reticulum in its germ-cells. Perroncito does not pay much attention to mitochondria ; his claim that a true reticulum exists in Paludina spermatocytes I reject. The Golgi rods or chondrioplasts do not fuse to form a reticulum. Perroncito invents a good word for the sorting out of the Golgi rods between the daughter-cells—"dictyokinesis"—on the analogy of the word "karyokinesis." "Dictyosome" and "Dittosome" are the words used by Italian writers for the Nebenkern or Golgi batonette, but be it noted that Murray (**33**) was the first observer to describe dictyokinesis; he used the snail, and his work is a valuable contribution.

A. The Correct Identity of the "Chondriosomes of Meves" and "Mitochondria of Benda" in Paludina.

What Perroncito calls the "chondriosomes of Meves" are undoubtedly the ordinary mitochondria, and I have called them so throughout this paper. The identity of the "mitochondria of Benda" is a difficult matter. They appear in the cytoplasm, as far as one can make out, pari passu with the spermateleosis stages, from a cytoplasmic condensation. In Pl. 25, fig. 20, and in other such spermatocytes, the cytoplasm appeared smooth. No staining method revealed these granules in spermatocytes or even in young spermatids. The question then arises as to whether these bodies are really mitochondrial ; it must certainly be mentioned that they stain

very like mitochondria. They are not, however, destroyed in corrosive acetic acid, and stain very densely with iron-hæmatoxylin after this fixative (**35**).

I am inclined to think that the granular bodies (mitochondria of Benda) are not true mitochondria, but may be in some ways chemically allied to the true mitochondria. More intense fixation and staining methods may possibly reveal them in earlier stages, but there is nothing in the typic series even remotely resembling the "mitochondria of Benda." It is possible that the granular bodies may be homologous with the "albuminous bodies" described by other authors for marine prosobranchs.

B. The Differentiation of the Two Kinds of Spermatozoa of Paludina vivipara.

I have shown that the two sorts of spermatozoa can easily be traced back to two sorts of spermatogonial cells, whose mitochondrial apparatus is quite different. I cannot feel quite so certain as to whether this difference exists among the primordial germinal epithelial cells.

It has been stated that the typic spermatogonia have rod-shaped mitochondria (chondriokonts), while the giant have granular spherical mitochondria (chondriosomes).

A word of warning must be written here with regard to this. We already know of cases where the mitochondria are able to be either granular or rod-shaped (vide Champy (**5**)). It is possible, though I think not very likely, that all germinal epithelial cells in Paludina have chondriokonts or rod-shaped mitochondria, and that this state persists when typic spermatogenesis is followed, and when the cells take the different path leading to the atypic state, the rods fragment and become granular. In the egg the mitochondria are nearly always fine and granular, and in other cases rod-like, but very fine, and this is undoubtedly connected with the metabolic processes carried out by the egg-mitochondria. Maybe, then, the atypic mitochondria become granular because of the peculiar meta-

bolic character of the atypic series; the latter cells become a good deal larger than the typic. It should, however, be mentioned that the main period of growth of the atypic series is during spermateleosis, i.e. the metamorphosis of the spermatid into the spermatozoon. We, however, have seen that at this period the mitochondria become clumped around the filaments, and evidently are not concerned in the growth of the spermatid. I do not mention here Perroncito's "chondriosomes of Meves," because their "mitochondrial" nature is a moot point.

A most remarkable fact which I wish to show here is that the growth-stage of the abnormal or atypic spermatocyte is, in a sense, the normal one; by this I mean to say that the atypic spermatocyte has a mitochondrial apparatus most like that in other animals. It is really the typic spermatocyte of Paludina that possesses the abnormal type—such enormous rods are rare; the vast majority of spermatocytes in the animal groups are characterised by mitochondrial apparatus just like that of the atypic spermatocyte of Paludina. This can easily be proven by reading the literature on the mitochondria (**4**, **24**, **7**, etc.). In the atypic spermatocyte we find the mitochondrial elements closely resembling those of Pulmonate Mollusca. One would hardly venture to conjure up the phylogeny of these molluscs to explain the occurrence of two kinds of sperms, because atypic sperms of this kind are not found elsewhere in the animal kingdom. Those of Lepidoptera are not homologous, as Meves would suggest.

The differences which exist between the atypic and the typic series in Paludina may be best seen in tabular form. It will then be understood that the typic spermatogenesis in so far as comparison with Pulmonate Mollusca goes is really the unusual one, in the early stages especially. Naturally, after the abnormal maturation divisions of the atypic series nothing homologous is to be found in snails of the Helicid type, but the earlier stages are almost exactly like those of Helix aspersa. Did we know the morphology and behaviour of the mitochondria of other groups of molluscs

	Primary spermatogonium.	Spermatocyte size (average).	Maturation prophases (nucleus).	Spermatocyte (full grown).
Paludina (typic) . .	Rest period after division short. Cytoplasm contains a good number of coarse rods near archoplasm	11 μ longest way	Rest, or dictyate stage absent; filaments never break up to form reticulum	Archoplasm about one-quarter size of that of atypic. Chondrioplasts few. Mitochondria rods number apparently about one dozen; more rarely about thirty spheres.
Paludina (atypic) . .	Rest periods after division not short, since cells do not immediately after division proceed to growth. Cytoplasm contains archoplasm with fine granular mitochondria	20 μ longest way	Dictyate stage present for a long time	Archoplasm dense, large, and chondrioplasts numerous. Mitochondria fine spheres; number generally several hundred.
Pulmonate . . .	Cytoplasmic inclusions like those of atypic series, or like those of Mammals, etc.	22 μ (Helix aspersa) longest way	Dictyate stage present as in atypic series	Archoplasm never quite so comparatively large as in atypic series, though chondrioplasts may be as numerous. Mitochondria as in atypic series of Paludina.

some interesting comparisons would be made possible. I only make the above suggestions tentatively, and do not wish them to be construed otherwise.

At the present time examination of the gametogenesis of a number of Molluscan families is being undertaken in order to ascertain, if possible which groups have the large elongate mitochondria of the typic sort in Paludina, and which are like Pulmonates in the possession of the usual granular mitochondria like those of the atypic series of Paludina. It is quite possible that such an organised examination of types related to Paludina and of types which might be within the phylogenetic line of the Prosobranchs might yield important evidence leading to an understanding of the true history of the atypic spermatozoa.

For the present I am uncertain as to what grounds might be brought forward for regarding the atypic spermatozoa as "survivals" of no present function. I am prepared to admit that this attractive view will need strong evidence to support it, and that it may even be quite impossible. At this stage I leave the matter till further researches have been carried out. I may say, however, that I am not attracted by the view that the typic spermatozoa represent the ova in the male Paludina that was once hermaphrodite like Helix.

Summary.

New Facts.—(1) In Paludina vivipara it has been shown that in the case of the well-known dimorphic spermatozoa the atypic (giant) cells have numerous fine granular mitochondria, while the typic cells possess a very few, large, stout, rod-shaped mitochondria.

(2) In the typic divisions it was thought that in some cases these large rods were merely sorted out into two groups, to the daughter-cells, while in other cases it was shown that the rods were divided in the middle.

(3) In the atypic divisions the mitochondria acted like those of Helix aspersa or other pulmonates.

(4) Spermateleosis stages in both atypic and typic spermatogenesis have been carefully followed out; the Golgi apparatus sloughs off in both series.

(5) In rare cases the mitochondria of the typic spermatocytes are very large, coarse granules, quite distinct from the smaller granules of the atypic series.

Note on the Golgi Apparatus ("Nebenkern") of Helix aspersa, etc.

Demoll's (9) statement that the appearance of the "Nebenkern" (Golgi apparatus) heralds in some way the differentiation of the indifferent cell into either spermatogonium or oögonium was shown to be wrong in my paper on Helix (15). Additional proof of the incorrectness of Demoll's views is provided by preparations of Helix ovotestis made by Kopsch's method. In these the Golgi apparatus or "Nebenkern" is found in the smallest and most indifferent germinal epithelial cells, being stained a dense black. In Text-fig. 21, *CH.*, I have drawn a part of the ovotestis wall from a Kopsch preparation showing the apparatus of Golgi in every cell. Demoll is therefore wrong in considering the Golgi apparatus (his "Nebenkern") has anything to do with the determination of oögonium or spermatogonium. In Pl. 25, fig. 28, a young spermatocyte is drawn from a Kopsch preparation. The Golgi apparatus alone stains black.

I have found this Golgi apparatus in cells of Paludina, Helix, Arion, and Limnæa among Mollusca.

Part II.—The Germ-Nurse Cells of Helix Aspersa, Testacella Haliotoïdes, etc. (Pl. 26.)

In Pulmonate Mollusca it is well known (2, 4) that some of the germinal cells grow to form ova, some spermatozoa, some follicle cells, some sertoli (sperm nurse-cells), and others large hypertrophied nurse-cells especially common near egg-cells, and full of yolk. These large nurse- or yolk-cells are derived from true germ-cells of the original germ rudiment, and belong

to the same series of cells as the other elements in the ovotestis. In my previous paper on Helix (**15**, p. 567), I have given a figure showing the typical nurse-cells of this form. In this animal the nurse-cells are distinctly hypertrophied and hyperchromatic, but rarely more than one and one-half times larger than the full-grown spermatocyte. In the case of Testacella the yolk-cell nucleus is generally at least four and one-half times the size of the nucleus of the full-grown spermatocyte; the same is apparently the case in Limnæa stagnalis, which I have not studied so carefully as Testacella.

In Pl. 26, fig. 29, is drawn a fairly typical nurse-cell containing a large number of yolk spheres at *Y.*, a Golgi apparatus at *G.A.*, and what are possibly to be identified as mitochondria at *M.* In the scheme in Pl. 26, fig. 37, on the bottom right-hand corner at *I.* is a yolk-cell in sitû, to which are sticking a large number of unripe spermatozoa (8). At *I.I.*, on the left bottom corner, is another yolk-cell with a partially disintegrated cytoplasm. The size of nucleus of the spermatocytes at 5, in the lower middle region of the scheme, may be compared with that of the yolk-cells. It will be seen that in cases the yolk-cell nucleus may be as large as the entire cell of the spermatocyte; the spermatocytes in Pl. 26, fig. 36, at 5, are drawn to scale exactly, as are all the other elements in this scheme.

In Pl. 26, fig. 28 bis, is drawn a progerminative germinal epithelial cell—that is, a germinal epithelial cell showing signs of passage to spermatogonium or oögonium; Pl. 26, fig. 29, below, is drawn to the same scale. In Pl. 26, figs. 31 and 33, are drawn two subsequent stages in spermatogenesis —early leptotene, and the bouquet or contraction stage (after synapsis). In Pl. 26, fig. 35, is a spermatid, and in Pl 26, fig. 36, the head of a ripe sperm. In the scheme in Pl. 26, fig. 37, is drawn to a much smaller scale every stage in spermatogenesis, the different stages from spermatogonium to sperm being marked by the Arabic numerals 1 to 9. No. 2 represents the growing spermatogonium, No. 3 the spermato-

cyte half-grown, No. 4 a three-quarter-grown spermatocyte, No. 5 a group of prophases and mitotic figures of the first maturation division, No. 6 second maturation division, No. 7 young spermatids, Nos. 8 and 9 older and nearly ripe spermatozoa. For elaborate drawings of some of these stages see my last paper (**16**). It will be noted, I trust, that all these figures in my scheme are convincing, and correspond with what is already known with regard to the comparative sizes of the elements of spermatogenesis.

At the capital letters, *A*, *B* and *C*, are drawn three oöcytes; the one at *A* is in the contraction figure; compared with the same stage in the sperm series at 3, below, it will be seen that there is a perfect correspondence in size between the two; *B* and *C* are later stages, but the egg grows much larger than that in *C*. All around the wall of the ovotestis (covered by Ancel's layer) are seen the indifferent germinal epithelial cells at *G.E.*

The above-described stages of normal spermatogenesis and oögenesis begin in spring and go on all through the summer and late autumn. Towards autumn the cavities or alveoli in the ovotestis become more or less completely cleared of both ripe eggs and sperms, which pass off to the hermaphrodite duct.

The yolk-cells do not move off, and they lie in the cavities of the ovotestis all through winter without undergoing much change.

Now in spring a wonderful process may begin. The arrival of favourable weather sets in action the factors which cause the new crop of eggs, sperms and nurse-cells to begin developing, and the old yolk-cells are influenced by these factors, and themselves try to develop into germ-cells. A glance at Pl. 26, figs. 30, 32 and 34, will show what remarkable cells are so produced; these are drawn to the same size as all the other elements on the left side of the plate.

Reference to the scheme on Pl. 26, fig. 37, will serve to show these points; all the stages of such cells—I will call them giant germ-nurse cells because they are really only

hypertrophied germ-cells—are marked by roman numerals from *I* to *X*. Such a nurse-cell as at *II*, bottom left side, passes on to a giant leptotene stage as in *III*, at the middle; the latter stage then passes on to a late leptotene as at *IV*, with filaments, which again passes to a peculiarly abnormal synaptene as at *V*; and then one may get such an abnormal form as at *VI* with basophil droplets, to which converge the filaments in their immediate region. Now degeneration rapidly sets in: at *VII* the cell is quite abnormal, and by the stage drawn in Pl. 26, fig. 34, a rupture may appear and the cell contents flows out, and the giant-cell gradually disintegrates. I never found any stage later than synaptene and contraction figure.

Comparisons of the various stages in my scheme on Pl. 26, fig. 37, will serve to show that I have established clearly that what I have described is correct—the three series, egg, sperm and nurse-germ cells are clearly marked, from alpha to omega.

Having given the broad outline of the facts concerning these peculiar cells, I will describe their structure.

By focussing up and down upon a germinal epithelial cell, just pro-germinative, one may see from ten to thirty rough chromatin blocks in the nucleus (Pl. 26, fig. 28 BIS). The nurse-cell nucleus contains blocks of much the same size and appearance, but these are enormously more numerous; the nurse-cell nucleus is in a state known as hyperchromacity (Pl. 26, fig. 29). Of all the elements of the ovotestis the nurse-cell nuclei are most darkly staining and conspicuous. Such nurse-cell nuclei, though quite distinct from the ordinary germinal epithelial cell, are united to the latter by a perfectly graduated series of intermediate stages; the germinal epithelial cell grows quickly, its nucleus becomes filled gradually with more and more chromatin blocks, which often stain more heavily than the original ones in the epithelial nucleus, the yolk in the cytoplasm becomes marked in quantity, till finally one gets the cell as drawn in Pl. 26, fig. 29, or Pl. 26, fig. 37, at *I*.

The above facts are clearly demonstrable, and it is

impossible to recognise when the epithelial germ-cell ends and the nurse-cell begins in the series. This is a very important fact.

In Pl. 26, fig. 37, at the bottom left corner at *II* and on the right at *I* are drawn very typical nuclei of nurse-cells; that in Pl. 26, fig. 29, is not quite typical, as will be noticed later. In the central region of the nucleus one finds a confused conglomeration of chromatin blocks seemingly representing a karyosome.

No sign of a plasmosome could be found. The chromatin blocks otherwise are set apart from one another, as shown in Pl. 26, fig. 29. In the latter the cell is showing the appearance of "pro-germinativeness"—that is, of attempting, at least, to become a developing germ-cell; the cell has broken away from the ovotestis wall (see Pl. 26, fig. 37, at *II*, where this is beginning to take place), there is a large basophil karyosome, and two ring-like bodies at *R.*, whose nature I could not ascertain. They are rings of basophil matter, enclosing a chromophobe material; at *G.A.* in the cytoplasm is a large archoplasm with accompanying Golgi elements (batonettes).

This stage represents for the germ-nurse cells the same stage in the epithelial cell above. Such peculiar cells float out into the liquid inside the ovotestis alveoli, and their yolk soon disappears. In the next stage, drawn in Pl. 26, fig. 30, the chromatin blocks are beginning to run together to form the well-known leptotene stage. In this nucleus are two large abnormal plasmosomes and a large double karyosome; the cytoplasm appeared to be quite clear of yolk, but at one side (below) in a juxta-nuclear position was to be seen a cytoplasmic zone. This cell lay in a region of the ovotestis where the cytoplasmic and deutoplasmic inclusions were not well preserved. Degeneration may also account for the absence of granular bodies in the cytoplasm. The corresponding stage in normal spermatogenesis is drawn in Pl. 26, fig. 31. The mitochondria form a heap in the juxta-nuclear, excentric position, in the same position as the cloud in Pl. 26, fig. 31.

COMPARISON BETWEEN NORMAL AND GIANT-CELLS.

Stage chosen for comparison.	Cell size.	Nuclear size and nuclear condition.	Chromaticity and number of loops.	Cytoplasmic elements.
Normal presynizesis (Pl. 26, fig. 33)—a little later	Circa 16 μ in diameter, longest way	Circa 14 μ	Chromaticity varies somewhat, but may be like that of giant-cell. Loops about 20 in number, fusing to form about 10 pairs	Mitochondria excentric, juxta-nuclear; Golgi apparatus generally, though not always, covered by mitochondria.
Large giant germ-nurse cell in same stage as above (Pl. 26, figs. 32, 34)	Circa 40 μ to 50 μ; in one case 60 μ	Circa 30 μ to 40 μ. Nucleus often contains pathological nucleoli or necrotic droplets	Chromatin, like that of normal stage, varies in staining, according to circumstances of fixation, etc. It is generally like the normal. Number of chromatin filaments or loops seems to exceed 20. Instead of 10 pairs there may be 30. Grouping of filaments abnormal	Mitochondria generally grouped as above. The Golgi apparatus not generally demonstrable, being covered by mitochondria. In cases latter a little larger than normal.

The next stage is one which reveals most remarkable pathological and necrotic forms. Droplets of chromophil matter appear in the nucleus in some cases, and the cells become so unhealthy that one may get the strange appearance as in Pl. 26, fig. 37, at *VI* in the middle of the scheme. Such huge "karyosomes" have radiating from them, like the spokes of a wheel, the chromatin filaments. In Pl. 26, fig. 32, a strange cell is drawn; it is a "paired filaments" stage just before synizesis and contraction, only so abnormal as to be unlike any stage of the normal spermatogenesis; at *X* is a peculiar hub-like body, containing a central slightly chromophil mass, a clear peripheral zone, a ring, and the usual radiating spokes like those of a wheel. There are three or four abnormal "karyosomes" or chromatic droplets in this nucleus. The cytoplasm of the cell contains a large number of clearly-defined mitochondria, somewhat larger than those of the normal cell (see Pl. 26, fig. 35), but disposed mainly in a juxta-nuclear, excentric position, as usual with this stage of the normal spermatogenesis.

In the scheme on Pl. 26, fig. 37, this same cell is drawn at the upper side at *V* for comparison with other cells, and at *IV*, to the right, is a late leptotene stage of the giant germ-nurse cell, the stage just before that in *V* and *VI*. The cell at *IV* is fairly healthy, and would have gone a good distance in its metamorphosis had external conditions been favourable.

The abnormal paired chromatin filaments now fuse, and one has the synaptene stage (Pl. 26, fig. 34). This cell contains a tripartite "karyosome," a meagre cytoplasmic juxta-nuclear cloud as in Pl. 26, fig. 30, and, what is more important, the cell-wall has burst or disintegrated at *B.R.*, and the cell would soon have broken up completely. I have found no giant germ-nurse cell to go much further than this stage, and the majority do not go so far. In Pl. 26, fig. 37, at *VII*, on the left, is drawn another cell with a completely abnormal spireme, and it is possible that this cell represents a pachytene stage just after the bouquet stage. If so, this is the furthest I have traced such cells. Pl. 26, fig. 37, *VII*, has no inclusions

in the cytoplasm. Such is the peculiar history of some nurse-cells.

Golgi Apparatus and General Morphology of the Giant Germ-nurse Cells.

In most cases the Golgi apparatus of the normal spermatogonium, after entry into the prophases of the heterotypic division is covered over by and indistinguishable from a mass of granular mitochondria; in other cases the Golgi apparatus is clearly to be seen. Just the same two conditions appear to old with the giant-cells. Pl. 26, fig. 32, in this way resembles Pl. 26, fig. 33, while the cell in Pl. 26, fig. 29, which has a clearly marked Golgi apparatus, would be like that in Pl. 26, fig. 37, at 3.

Cell size and nuclear size also correspond comparatively in giant- and small cells. Compare Pl. 26, figs. 30 and 31, figs. 32 and 33, and figs. 33 and 34, where it will be noted that a remarkable correspondence in not only size, but in the excentric position of the nucleus in the cell, can be seen to be the case; this, as noted before, applies also to mitochondria. In a few cases the cytoplasm of the giant-cell is too large in comparison with the nucleus (using the normal cell as basis for this comparison), and such cells are found degenerating as well as those in which the nucleo-cytoplasmic ratio is the same as in the normal cell.

Number of Chromosomes in Normal Testacella Germ-cells, compared with the Number of Pachytene Loops in the Giant-cells.

In my material, nearly all of which is fixed in chromosmium, it is difficult to count the number of chromosomes. I believe the number of somatic chromosomes is over twenty, the haploid number about ten. In the normal cell at pachytene I count about ten or twelve loops; in the giant-cell, by the same method of counting, I have found from twenty-five to thirty; in one clear case, where a giant-cell and a normal

cell lie side by side in the late bouquet stage, the filaments in each cell are the same thickness, while the nucleus of the giant-cell is literally a dense, tangled mass of filaments; the giant-cell contained twice as many filaments in one section as the normal cell, and the latter only appeared in two sections, while the giant-cell was cut into four sections, and three of the sections contained pieces of nucleus with many tangled filaments. This, however, is not complete proof that were the chromosomes to appear they would be more numerous than in the ordinary germ-cell, for the filaments in the giant-cell might merely be longer than in the normal cell and more coiled therefore. This seems to me to be unlikely, and the evidence seems to show that the filaments are more numerous in the giant-cell than in the other.

Fate of Intermediate Forms between Nurse-cells and Germinal Epithelial Cells.

It has been stated that there is a complete chain of forms intermediate between nurse- and germinal epithelial cell, because the latter gives rise to the former. Every stage between the small germinal epithelial cell and the giant-cells can be found at any time of the year. As has been explained, the larger the nurse-cell the more chromatin lumps there are for the nucleus and the more yolk-disclets in the cytoplasm. There is no true difference between the giant-cell and the small one, as has been shown to be the case in man **31**) (see Montgomery), where a special sertoli or nurse-cell determiner is segregated into those cells destined to form sertoli cells. Careful observation of nurse-cells in Helix and Testacella failed to show any such body in nurse-cells.

Intermediates as above described obviously vary in the distance they have gone on the path of differentiation, and equally, therefore, vary in the capacity they show when they are stimulated to de-differentiate. Many cells somewhat larger than the full-grown spermatocyte appear to succeed completely in de-differentiating to the same size as the spermatocyte

and finally to undergo normal stages in the prophases of the heterotypic division. In Pl. 26, fig. 37, at *III*A and *V*A are three cells, all of which were a size which would allow them to recover their equilibrium and so de-differentiate successfully. Compare *III*A with the cells at 3, or the right in the same stage.

In some cases these intermediate cells, even though small enough to pass easily to the prophases of the heterotypic division, are abnormal in appearance. This abnormality is seen in the coarseness and number of chromatin filaments in their nuclei. Both *V*A and *III*A have nuclei unlike the normal stage.

Germ-nurse Cells in other Molluscs.

Such large cells have been found in profusion in Helix aspersa, and I have little doubt they occur in Limnæa and other molluscs I have studied (16). In Helix the yolk-cell nucleus does not grow relatively to the spermatocyte so enormously, and consequently there is a good deal of difficulty in establishing the facts so clearly as for Testacella. Should this description be doubted I will be prepared to publish microphotographs to establish what I claim. No one can study the ovotestis of Helix aspersa without finding large, often naked nuclei lying free in the lumen, mixed up with sperm-cells. These nuclei regain a cytoplasm, and their dense hyperchromatic nuclei gradually change till they resemble those of the ordinary stages of spermatocytes. I have carefully studied them in Helix, and have found that many germ-nurse cells do de-differentiate and finally form spermatozoa.

Germ-nurse Cells—Oöcytes or Spermatocytes?

So far I have compared the giant-cells exclusively with sperm stages. The question then arises: Would the germ-nurse cells have become spermatocytes or oöcytes had they succeeded in developing? Naturally one might consider them

to be likely to become oöcytes because of their yolk, but since they fall into the lumen of the ovotestis and do not stick on to the walls like the oöcytes, they are certainly exposed to two sources of stimuli: besides the yolk-disclets, whose presence must exert some stimulus on the giant-cell, there is the fact that only stages in spermatogenesis are found in the ovotestis lumen, and one might expect that any cell carried into this locality might be affected by the special conditions existing in the lumen of the ovotestis. As has been remarked before (**15**), the cell is outwardly indifferent until it passes beyond the pachytene stage, so that it is not generally possible in Pulmonate Mollusca to identify a cell as spermatocyte or oöcyte during the prophases of the heterotypic division. In the smaller germ-nurse cells, which I believe de-differentiate successfully, the further stages after escape of the cell from the germinal epithelium leads to the formation of spermatocytes. Evidently the cell can be indifferent up to quite a late stage.

Summary on Testacella Germ-nurse Cells.

(1) Germinal epithelial cells, besides producing ova, spermatozoa and follicle cells, may also become much enlarged to form yolk- or nurse-cells, which have very large hyperchromatic nuclei and a cytoplasm full of yolk-discs.

(2) Such cells are easily distinguishable from stages of spermatogenesis, because of their large size, and often because of their abnormal appearance.

(3) In spring and summer normal spermatogenesis and oögenesis goes on, the ova and sperm-cells being nourished by the nurse- or yolk-cells. Towards autumn and winter the cold weather stops such activity, and by this time the ovotestis cavities are nearly vacant, because the ripe products have passed away through the genital duct. The large yolk-cells are left, often exhausted more or less of their yolk-discs, and show signs of falling away from the ovotestis wall to which they previously adhered; they float free in the fluid

contained in the partly empty ovotestis alveoli or lumina, and in many, though not all cases, their cytoplasm partly breaks up.

(4) During winter the Testacella hibernates and all activity is possibly suspended. In the early spring following activity in the ovotestis recommences, and germinal epithelial cells are stimulated to begin proliferating series of egg and sperm-cells. These activating materials (stimuli of some sort) affect not only the above cells but reach to the giant nurse-cells, which begin to undergo the prophases of the heterotypic division, known as leptotene, synaptene, contraction figure, pachytene and diplotene. Such large cells have been found to pass more or less abnormally through all the stages up to pachytene, but about this stage they degenerate. In the majority of cases these nurse-germ cells do not arrive at such a late stage, and many others possibly degenerate very early.

(5) In a scheme on Pl. 26, fig. 37, I have drawn all the elements found in the ovotestis at any time of the year, and in Pl. 26, figs. 28–36, I have drawn carefully examples from both normal male germ-cells and from the giant germ-nurse cells.

(6) The number of chromosomes in Testacella seems to be something over twenty and the haploid number over ten, probably about twelve. The giant germ-nurse cells are found to contain at the synaptene stage too many loops, and this is the case in the pachytene stage; normal pachytenes have some ten loops, the giant-cells apparently as many as twenty-two to thirty, but in no case could I be quite certain as to their number. I feel sure that the giant germ-cells contain an irregular and over numerous series of chromatin loops. Moreover, the nuclei of the giant-cells generally contain many droplets of a chromatoid nature, as well as pale spheres, which are not found in the normal spermatogenesis stages.

Discussion with Regard to the Giant Germ-nurse Cells.

The above summary sufficiently explains the salient points in my researches on the peculiar nurse-germ cells.

The main questions which this part of my paper raises are as follows: What is happening in the germinal epithelial cell as it becomes hyperchromatic? Is the new matter which comes into the nucleus true chromatin? Why cannot the giant-cells succeed in passing through the prophases and form giant chromosomes? What are the chromophil droplets which appear in the nucleus of the giant-cells just before they begin finally to degenerate? If these droplets are chromatin, does such chromatin differ from that still left in the filaments or spireme?

These questions are very difficult to answer, and reach to the root of the various controversies on how. chromatin grows, and what limits the size of the cells and body of any animal or plant. Apart from the nurse-cells of Pulmonate Mollusca, one finds hypertrophied, hyperchromatic cells in many animals: in insects such as Coleoptera and Hymenoptera the ovarioles contain large nurse-cells; in the trophoblast of such a mammal as the mouse and in other cells where there is a storage or constant exchange of food-materials the cells enlarge, and they become very rich in "chromatin." Written in a few words, the whole train of occurrences in the giant-cells of Testacella seems to be that the amount of chromatin increases step by step with the formation of yolk, and when the function of the cell temporarily lapses, the latter tries to recover its equilibrium by shedding the superfluous matter in the form of drops, generally fails, and then undergoes disintegration. Nucleo-cytoplasmic relationship may have something to do with this question, but I have no evidence suggesting that were the cytoplasm of the giant-cell larger the latter would not then lose its equilibrium and perish.

Differentiation may be the key to the problem; differentiated

for nutrition, the giant-cell is stimulated, endeavours to recover the property of metamorphosis into a sex-cell, and fails to do so—not possibly always because its internal condition inhibits this, but because the competition in the ovotestis becomes rapidly great as the new cells develop, and the unwieldy over-differentiated one is choked out by the normal rapidly-growing sperm and egg-cells. To use Child's word, the giant-cell tries to " de-differentiate," but fails (6).

It is not desirable to enter seriously at present into the possible view that the binuclearity hypothesis (one of these hypotheses) would serve to explain these phenomena. It might be supposed that the nurse-cell became charged with trophochromatin, and that the appearance of droplets of chromatoid matter in the giant-cell was to be interpreted as an attempt by the latter to throw out its trophochromatin in favour of its idiochromatin. This view, specious as it is, may be worthy of examination and criticism. I neither uphold nor condemn it at present.

Finally, the stimulant which arises in the body of the hibernated animal at spring, and which causes the giant-cell to attempt de-differentiation and metamorphosis, may be identified with some physiological secretion or hormone caused somehow by the changed weather conditions. This phenomenon is no new fact; but that such a hormone should have an effect on a highly differentiated cell like the nurse- or yolk-cell is remarkable, and serves to show that a very highly differentiated cell may, provided certain stimuli be forthcoming, enter upon an attempt to de-differentiate and so prepare tself for a new cellular function.

June, 1918.

Summaries.

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DESCRIPTION OF PLATES 25 AND 26.

Illustrating Mr. J. Bronté Gatenby's "Notes on the Dimorphic Spermatozoa of Paludina, and the Giant Germ-nurse Cells of Testacella and Helix."

Lettering to Plate 25.

AF. Axial filament, or filaments. *A.L.* Ancel's layer. *A.M.* Archoplasm with Golgi apparatus (*C.P.*), and sometimes with mitochondria. *A.X.* and *B.X.* Regions in the forming axial rod (*R.D.*). *C.* Centrosome or centrosomes. *CH.* Chromosome. *CP.* Chondrioplast or Golgi (Nebenkern) rod. *G.B.* Granular bodies of unknown nature, "mitochondria of Benda." *G.M.* Granular mitochondria. *H.* Chromatin (?) head of atypic sperm. *M.* Mitochondria. *M.R.* Mitochondrial rod. *M.S.* Mitochondrial sphere. *M.R.s.* Mitochondrial rods tangled. *M.R.x.* Mitochondrial rod especially clear. *N.* Nucleus. *R.D.* Central rod of atypic sperm. *Y.*, *Y.D.* Yolk discs.

Lettering to Plate 26.

B.R. Break in cell wall indicating early disintegration of cell. *G.A.* Golgi apparatus (Nebenkern). *K.* Karyosome. *M.* Mitochondria. *N.* Nucleus. *R.* Chromatin ring. *X.* Abnormal wheel-like structure in giant pathological cell.

For letters in Pl. 26, fig. 37, see description of that figure on page 423.

[Figures drawn either with a 12 or an 18 comp. eyepiece and a $\frac{1}{12}$ semi-apochromatic Koritska oil-immersion objective, and then somewhat reduced. Scale given at middle of plates. In certain spermateleosis stages of the atypic series the chondrioplasts have been added to figures of Flemming-without-acetic iron-hæmatoxylin preparations, from material treated by Kopsch's method.]

PLATE 25.

[With the exception of figs. 25 to 28, all the figures on this plate are drawn to the scale given in the middle of the plate from preparations made in Flemming-without-acetic acid and iron-hæmatoxylin. Figs. 27 and 28 are drawn from Kopsch material. Figs. 25 A and B are at half the magnification of the other figures.]

Fig. 1.—Secondary spermatogonium of typic series just after entry to the growth stage. The archoplasm, chondrioplasts and rod-shaped mitochondria are shown. At least seven mitrochondria could be counted.

Fig. 2.—View of a cell at the same stage as the previous figure, looking down upon the archoplasm and the surrounding mitochondrial rods. There appeared to be at least thirteen of the latter, but it was not possible to say whether or no the individual U-shaped rods were separate.

Fig. 3.—Full-grown typic spermatocyte, before syndesis, and some time before the chromatin filaments condense to form chromosomes. The mitochondrial rods are larger, longer, and in some cases straighter. There were at least nine.

Fig. 4.—Polar view of equatorial chromosome plate (seven chromosomes) of the first maturation division. At least seven mitochondrial rods could be counted. These had become thinner and more elongate, and their enumeration had become very difficult because they were S- and U-shaped, and therefore never quite in the same focus. The cell in fig. 4 is a very clear example.

Fig. 5.—Second maturation division metaphase showing mitochondrial rods. There appeared to be nine.

Fig. 6.—Second maturation division telophase showing characteristic grouping of rods. There were apparently no fewer than seven.

Fig. 7.—Second maturation telophase showing constriction of rods at equator of cells (*C.M.R.*). In each cell there were four rods.

Fig. 8.—Spermatid after re-formation of nucleus. Rods four in number, and are beginning to contract up, preparatory to becoming spheres, as in the next figure.

Fig. 9.—Side and end view of spermatid after the rods have become spheres. In the upper cell the archoplasm and chondrioplasts are clearly seen; the spheres are four in number.

Fig. 10.—End view of a second maturation division telophase to show mitochondrial rods; for a description see page 409.

Fig. 11.—Spermatid after the mitochondrial spheres have fused together to form the macromitosome. The acrosome has just been formed in connection with the archoplasm, which has now drifted aside. (Compare Text-fig. 9.)

Fig. 12.—Archoplasm and part of cytoplasm sloughing off, while macromitosome has elongated.

Figs. 13 and 14.—Two cells from the germinal epithelium to show the rod-shaped mitochondria (*M.R.x.*, *M.R.x.*, *M.R.*). (Compare with the cells in Pl. 25, fig. 17, where there are no rod-shaped mitochondria clearly to be seen.

Fig. 15.—A spermatogonial cell which did not contain elongate mitochondria, and which was supposed to be of the atypic series.

Fig. 16.—A spermatogonial cell in division, polar view, showing rod-shaped mitochondria.

Fig. 17.—Germinal epithelial cells apparently not containing rod-shaped mitochondria, and therefore supposed to belong to the atypic series.

Fig. 18.—Young atypic spermatocyte showing granular mitochondria. Corresponds with the typic cell in fig. 1.

Fig. 19.—Atypic spermatocyte nearly full grown. Archoplasm and chondrioplasts much larger. Latter more numerous than in the corresponding stage of atypic series (fig. 2).

Fig. 20.—Prophase of first maturation mitosis of atypic cell. Mitochondria and chondrioplasts sorted out into two heaps around the two centrosomes. Compare cell bulk with that of the typic series in fig. 4. Cytoplasm smooth.

Fig. 21.—Second maturation division of atypic series. The fourteen chromosomes lie haphazardly generally in a paler median region of the cytoplasm. At *C.P.* are the two groups of chondrioplasts which were added to this and the subsequent figures (21–25) from observations made on Kopsch material. The mitochondria, *G.M.*, are scattered around the paler central region of the cytoplasm. (Compare with the typic second maturation division in fig. 6.)

Fig. 22.—Atypic spermatid. Two nuclei have become re-formed, while at *C.H.* are several of the other chromosomes degenerating; the mitochondria are grouped at *G.M.*, the chondroplasts at *C.P.* In the cytoplasm is gradually appearing definite patchy regions, which later become more darkly staining and form the "mitochondria of Benda" (*G.B.*).

Fig. 23.—A later stage. The axial filaments, some twelve to fourteen in number, which began to grow out in fig. 21, are now quite long, but they grow even longer. In the cell the chondrioplasts are separated into two groups.

Fig. 24.—Atypic spermatid for comparison with the typic spermatid in fig. 11. At *N.* is the modified nucleus, at the back of which is attached the axial filaments. At *C.P.* is a mass formed of chondrioplasts. The granular bodies (*G.B.*), or "mitochondria of Benda," are much clearer. Some (possibly) of the ordinary mitochondria lie in the axial rod (*A.X.*) in front, but most lie behind at *G.M.*

Fig. 25.—Half the magnification of the preceding figures. Shows in A the typic, and in B the atypic spermatozoon; in both cases the sperm is not quite ripe. Before A becomes mature the bodies at *C.H.* and *C.P.* are sloughed off, and the entire length of the sperm becomes nearly of an even bore. The "mitochondria of Benda," or what

preferably are to be called the granular bodies (*G.B.*), are very chromophile.

Fig. 26.—A part of the middle region of a ripe atypic spermatozoon drawn at twice the magnification of the preceding figures to show the granular bodies (*G.B.*) and the axial rod (*R.D.*).

Fig. 27.—The head of a typic sperm to show the spiral twist (Kopsch).

Fig. 28.—Kopsch preparation of an atypic spermatocyte to show the way in which the chondrioplasts (Nebenkern or Golgi-apparatus) alone stain darkly.

PLATE 26. (Lettering, see p. 439.)

[All figures on left side of plate from fig. 28 BIS to fig. 36 drawn to scale indicated in middle of plate. All figures in the scheme in fig. 37 drawn to same scale indicated on right side of plate. Figures drawn from preparations stained and fixed in iron-hæmatoxylin and Flemming without acetic acid or diluted Champy.]

Fig. 28 BIS.—Young progerminative germinal epithelial cell of Testacella.

Fig. 29.—Dislodged slightly abnormal yolk or nurse-cell of Testacella. The dark karyosome is unusually spherical and noticeable; the Golgi apparatus is also very clear, and the cytoplasm is full of yolk-discs (*Y.*).

Fig. 30.—Giant germ-nurse cell in early leptotene stage; contains two large abnormal "plasmosomes," staining palely. Cytoplasm almost clear except for excentric juxta-nuclear cloud marking position of ill-stained or fixed mitochondria.

Fig. 31.—Corresponding stage in normal spermatogenesis.

Fig. 32.—Pathological giant stage of "paired threads" or diplotene stage; the threads converge towards, and are partly covered by, a mass of mitochondria (*R.G.A.*). There are several abnormal karyosomes (*K.*), while at *X.* is a peculiar wheel-like structure of an unusual nature, possibly representing a forming or partially absorbed karyosome (see Pl. 26, fig. 37, *VI*, in middle). The normal stage corresponding has nothing like this body at *X.* Compare for size with normal (slightly later) stage in fig. 33.

Figs. 33 and 34.—Giant and normal bouquet stages. Fig. 33 has some ten or eleven loops, fig. 34 about twenty-six, and this cell was cut into three sections. Karyosome in fig. 34 abnormal, tripartite instead of bipartite. At *B.R.* the cell has begun to disintegrate, and the excentric cytoplasmic mass is abnormal.

Fig. 35.—Early spermatid for comparison with other stages.

Fig. 36.—Head of ripe sperm of Testacella.

Description of Scheme in Fig. 37.

This elaborate figure represents the ovotestis of Testacella haliotoïdes for all times of the year. With the exception of Nos. *I* and *II*, the cells indicated by Roman numerals are never found except in late autumn or early spring. All the other elements are found throughout the year in both winter and summer.

A, *B* and *C* represent three stages of oögenesis, *C* being still small.

The Arabic numerals from 1 to 9 are stages in the normal spermatogenesis described on page 423 and in my previous paper (**16**).

Other letters are: *G.E.* Germinal epithelium. *A.L.* Ancel's layer (fibrous wall of ovotestis). *T.* Trabeculæ or folds in wall of ovotestis, forming pockets. *K.* Rejected karyosome, fig. *VII*.

Roman letters from *II* to *VII* represent stages in metamorphosis of giant germ-nurse cells. (Normal nurse-cell at *I* on right bottom corner.) *X.* represents giant-cells degenerating at an early stage. The cell *I.D.* on the left is degenerating in sitû. For more complete description of cells at Roman numerals see pages 425.